Molecular Profiling of Infiltrating Urothelial Carcinoma of Bladder and Nonbladder Origin☆

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Abstract

Advanced urothelial carcinoma (UC) has a poor prognosis and new treatments are needed. In our study, multiplatform molecular profiling of UC identified unconventional treatment options in most cases. Different molecular profiles are exhibited between bladder and nonbladder UCs.

Background: Infiltrating UC represents the second most common genitourinary malignancy. Advanced UC has a poor prognosis and new treatments are needed. Molecular profiling of UC might identify biomarkers associated with targeted therapies or chemotherapeutics, providing physicians with new treatment options. **Materials and Methods:** Five hundred thirty-seven cases of locally advanced or metastatic UC of the bladder, 74 nonbladder, and 55 non-urothelial bladder cancers were profiled using mutation analysis, in situ hybridization, and immunohistochemistry assays for biomarkers predictive of therapy response. **Results:** Molecular profiling of UC showed high overexpression of topoisomerase 2α, common phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha and/or phosophatase and tensin homolog (*PTEN*) alterations in nonbladder (27%) and bladder UC (21%), and rare gene mutations across subtypes. Compared with nonbladder, bladder UC consistently exhibited more frequent abnormal protein expression, including HER2 (10% vs. 3%; $P = .04$), tyrosine protein c-Kit receptor kinases (11% vs. 5%), c-Met proto-oncogene, receptor tyrosine kinases (25% vs. 8%), androgen receptor (16% vs. 6%), O(6)-methylguanine-methyltransferase (63% vs. 43%), ribonucleotide reductase M1 (32% vs. 11%), Serum protein acidic and rich in cysteine (SPARC) (69% vs. 33%), and topoisomerase 1 (63% vs. 39%). Bladder UC also exhibited increased amplification of *HER2* (12% vs. 2%; $P = .06$). **Conclusion:** Comprehensive molecular profiling of UC identified a large number of biomarkers aberrations that might direct treatment in conventional chemotherapies and targeted therapies, not currently recommended in this population. As a group, bladder UC exhibited higher levels of actionable biomarkers, suggesting that UC from different primary sites and non-UC are driven by different molecular pathways. These differences could have clinical implications resulting in different treatment regimens depending on the site of origin of UC.

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Introduction

Infiltrating urothelial carcinoma (UC; "transitional cell carcinoma") is the most common variant of urinary cancer, represents the most common genitourinary malignancy after prostate,[1] and is the fourth most common cancer in men.[2] Most UCs originate in the bladder but rare cases arise in the urethra, ureter, prostate, or renal pelvis. Approximately 25% to 30% of the patients with UC present with muscle-infiltrating tumors with substantial potential for further progression, metastases, and death.[3-5]

Previous molecular studies have shown that UC is characterized by mutations and losses of important cancer genes including Fibroblast growth factor receptor 3 (*FGFR3*), *ras family small GTPase proteins (RAS)*, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (*PIK3CA*), *retinoblastoma tumor suppressor protein (RB1),* phosophatase and tensin homolog (*PTEN*), and *tumor suppressor protein p53 (TP53).*[6-9] *FGFR3* and *PIK3CA* mutations are more prevalent in low-stage carcinomas,

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whereas *TP53* mutations are more common in muscle-infiltrating UC.[10] Mutations of epidermal growth factor receptor (*EGFR*) and *HER2* have been rarely observed,[11] but *HER2* amplification has been described in a subset of bladder cancer patients.[12,13] Neither comprehensive simultaneous analysis of these targeted pathways nor comparison of nonbladder versus bladder UC has been performed.

In the present study we investigated a broad panel of the most common targetable biomarkers in a large series of muscle-infiltrative and metastatic UC to identify treatment options that are not typically considered in this population. Patients with advanced UC are still not routinely offered individualized targeted therapeutic options based on tumor profiling[14]; National Comprehensive Cancer Network (NCCN) guidelines are limited to traditional chemo- and nontargeted therapies. The demonstration of the high frequency identification of actionable targets in UC patients' tumors might encourage more molecular profiling, potentially improving patient outcomes.

Materials and Methods

Tissue Samples

Test results of consecutive tissue samples (2008-2013) of locally advanced and/or metastatic UCs submitted to a commercial molecular profiling laboratory (Caris Life Sciences, Phoenix, AZ) were reviewed. Multiplatform profiling included immunohistochemistry (IHC), in situ hybridization, and sequencing.

Immunohistochemistry

Immunohistochemistry analysis was performed on formalin-fixed paraffin-embedded tumor samples using commercially available detection kits, automated staining techniques (Benchmark XT, Ventana, Tucson, AZ; and AutostainerLink 48, Dako, Carpinteria, CA), antibodies against androgen receptor (AR), topoisomerases 1 and 2α (TOPO1, TOPO2α) (Leica Biosystems, Buffalo Grove, IL); estrogen receptor (ER), progesterone receptor (PR), MET proto-oncogene, receptor tyrosine kinase (c-Met, human epidermal growth factor receptor 2 (HER2) (Ventana, Tucson, AZ); tyrosine protein c-Kit receptor kinase (c-Kit), epidermal growth factor receptor (EGFR), phosophatase and tensin homolog (PTEN) (Dako), O(6)-methylguanine-methyltransferase (MGMT), P-glycoprotein (PGP), thymidylate synthase TS) (Invitrogen, Grand Island, NY); transducin-like enhancer of split 3 (TLE3; Santa Cruz, Santa Cruz, CA); ribonucleotide reductase M1 (RRM1) (Protein Tech, Chicago, IL); Serum protein acidic and rich in cysteine (SPARC, monoclonal, R&D Systems, Minneapolis, MN; polyclonal, Exalpha, Shirley, MA) and tubulin beta-3 chain (TUBB3; Covance, Madison, WI). Scoring system and cutoffs for all antibodies are provided in Supplemental Table 1.

In Situ Hybridization

Fluorescent in situ hybridization (FISH) was used for evaluation of the *HER2* (*HER2*/CEP17 [chromosome 17 centromere] probe), *EGFR* (*EGFR/CEP7* probe), *TOPO2A* (*TOP2/CEP17* probe), and *c-MET* (*c-MET/CEP7* probe; Abbott Molecular/Vysis, Abbott Park, IL). *HER2/CEP17* ratio > 2.2 was considered amplified (based on guidelines from the College of American Pathology [CAP]/ASCO [American Society of Clinical Oncologists] 2007). *EGFR* amplification was defined as *EGFR/CEP7* ratio ≥ 2, or ≥ 15 *EGFR* copies per cell in ≥ 10% of analyzed cells. *TOPO2A* amplification was defined as *TOPO2A/CEP17* ratio ≥ 2.0 and *c-MET* was considered amplified if ≥ 5 *c-MET* copies were detected on average.

HER2 and *c-MET* status were evaluated using chromogenic in situ hybridization (INFORM HER2 Dual ISH DNA Probe Cocktail; commercially available *c-MET* and chromosome *7 DIG* probe; Ventana). The same scoring system was applied as for FISH.

Mutational Analysis

Next-Generation Sequencing. Direct sequence analysis was performed on genomic DNA isolated from formalin-fixed paraffin-embedded tumor samples using the Illumina MiSeq platform (La Jolla, CA). Specific regions of 45 genes of the genome were amplified using the Illumina TruSeq Amplicon Cancer Hotspot panel (Supplemental Table 2).

Sanger Sequencing. Mutation analysis using Sanger sequencing included selected regions of V-raf murine sarcoma viral oncogene homolog B (*BRAF*), Kirsten rat sarcoma viral oncogene homolog (*KRAS*), *c-KIT, EGFR,* and *PIK3CA* genes and was performed using M13-linked polymerase chain reaction (PCR) primers designed to amplify targeted sequences. PCR products were bidirectionally sequenced using the BigDye Terminator v1.1 chemistry (Applied Biosystems, Grand Island, NY), and analyzed using the 3730 DNA Analyzer (Applied Biosystems). Sequence traces were analyzed using Mutation Surveyor software v3.25 (Soft Genetics, San Francisco, CA).

Statistical Methods

Cohen κ was performed to determine the interrater agreement for 2 raters. The 2-tail Fisher exact test was performed to test where proportions of positive results were different according to tumor type ($P \leq .05$). JMPv10.0 (SAS Institute Inc, Cary, NC) and R v2.15 (R Foundation for Statistical Computing, Vienna, Austria) were used for statistical analysis.

Results

Clinicopathologic Characteristics of the Cohort

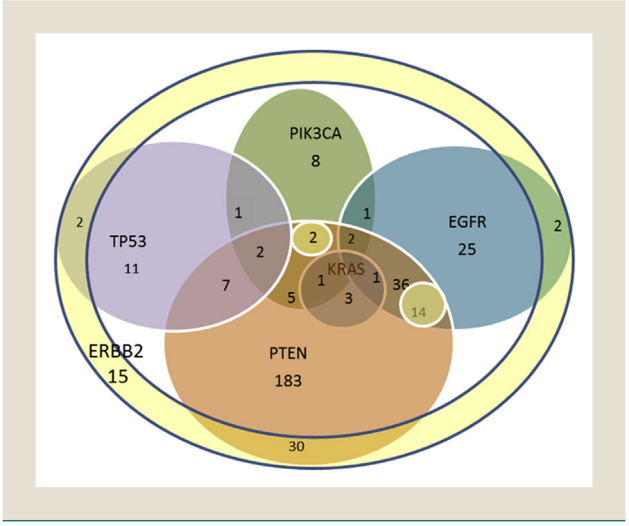
Five hundred ninety-two urinary tract carcinomas included 463 cases (87.5%) of bladder UC, 74 cases (12.5%) of nonbladder UC (renal pelvis, ureter, urachus, urethra), and 55 other histotypes (27 adenocarcinomas, 17 squamous cell carcinomas, and 11 small cell carcinomas).

Men constituted most patients (379, 64%) in every histologic category. The mean age of all patients was 66.3 years. Fifty-nine percent (273) of bladder UC cases were sampled at the primary site, and 41% (190) were from metastatic sites (lymph node and distant metastases). Nonbladder UC cases were similarly distributed (47 patients [64%] primary vs. 27 patients [36%] metastatic cases).

Profiling of the UCs for Clinically Actionable Markers

An immunohistochemical and molecular survey of UC was performed to evaluate concurrence of predictive biomarkers, revealing differential overlap of *TP53, PIK3CA,* and *PTEN* (Figure 1). The IHC and sequencing results are summarized

Figure 1 Associations and Frequencies in a Subset of Genes With Mutations, Copy Number Increases, and Changes in Protein Expression. Total Cases are Shown, With Coexisting Associations Indicated



(Tables 1 and 2, and Supplemental Tables 2-4). Most cases exhibited overexpression of 3 or more proteins and/or DNA mutations and amplifications, as described herein. No differences in biomarker expression between primary and metastatic sites (lymph nodes, distant metastases) were noted.

The EGFR and HER2 Pathway (Anti-EGFR and Anti-HER2 Therapies)

Epidermal growth factor receptor protein overexpression in bladder UC was seen in 41 of 53 (77%) cases; *EGFR* amplification was seen in 44 of 198 (22%) cases. One *EGFR* amplified case also exhibited a novel *EGFR* mutation (H773dup [kinase domain, exon 20]). A different *EGFR* mutation was identified in 1 nonbladder UC (770D_771NinsG). The frequency of *EGFR* amplification was greater in *HER2*-amplified (6 of 17; 35%) than non−*HER2*-amplified bladder UC (18 of 108; 17%; $P = .09$). Two of 4 nonbladder UCs overexpressed EGFR; 3 of 31 had *EGFR* amplification.

In bladder UC, HER2 protein overexpression (score 3+) was observed in 45 of 441 cases (10%) and gene amplification was seen in 33 of 284 (12%). Overall, IHC/ISH concordance rate for HER2 status was 91% ($\kappa = 0.589$). Only 1 UC case harbored a *HER2* mutation (missense, D769H, kinase domain, exon 19) and a *HER2* amplification. *HER2* amplification was also observed in 1 of 43 nonbladder UCs. In HER2-amplified bladder UC, additional genetic alterations such as mutations of *PIK3CA* were rare (1 of 8); *PTEN* and serine/threonine-specific protein kinase B (AKT1) alterations were not observed. Apart from the positive association with *EGFR*, no other biomarker showed significantly different status in regard to *HER2* amplification.

Status of c-MET (Anti−c-MET Clinical Trials)

Receptor expression of c-Met was observed in 31 of 123 (25%) bladder UCs, but *c-MET* gene amplification was found in only 1 of 75 cases. This case also harbored a *HER2* amplification. In contrast, nonbladder UC rarely overexpressed c-MET (2 of 24, 8.3%).

Status of c-KIT (Imatinib Therapy)

Expression of c-Kit was seen in 33 of 312 (11%) of the bladder UCs, nonbladder UCs exhibited a low rate of positivity (2 of 43; 5%). The *c-KIT* mutation was seen in only 1 bladder UC, a G565V, pathogenic substitution-missense mutation that has not been previously reported in bladder UC.

Table 1 Comprehensive Immunohistochemical Surveys of Urinary Bladder Carcinoma Subtypes

Histotype/IHC Marker (# tested)	UC, Bladder, %	UC, Nonbladder, %	Adenocarcinoma, %	Small-Cell Carcinoma, %	Squamous Cell Carcinoma, %
AR (545)	16.2	6.2[a]	3.8	11.8	9.1
ER (542)	1.9	1.6	0.0	0.0	27.3[a]
PR (541)	2.6	1.6	4.0	17.6[a]	0.0
c-Kit (387)	10.3	4.8	11.8	37.5[a]	0.0
c-MET (167)	25.2	8.3	44.4	0.0	0.0
EGFR (61)	77.4	50.0	0.0	0.0	100.0
HER2 (559)	10.2	3.1[a]	3.8	0.0	0.0
MGMT[b] (542)	62.9	43.3	69.2	20.0[a]	40.0
TLE3 (181)	23.9	11.1	22.2	0.0	33.3
TOPO2α (459)	68.1	71.9	76.2	92.3	57.1
TUBB3[b] (87)	39.0	23.5	80.0	80.0	0.0
PTEN (543)	63.0	56.7	37.5	25.0	25.0
PGP[b] (455)	27.2	15.5	45.5	7.7	11.1
RRM1[b] (513)	32.1	10.8[a]	33.3	62.5[a]	22.2
SPARC (303)	68.7	33.3[a]	19.2[a]	35.3[a]	9.1[a]
TOPO1 (493)	63.3	38.7[a]	32.0[a]	81.3	44.4
TS[b] (506)	17.0	22.2	12.5	47.1[a]	11.1

Abbreviations: IHC = immunohistochemistry; UC = urothelial carcinoma.
[a]Indicates significantly ($P < .05$) different expression between bladder UC and other histotypes.
[b]Expression of the biomarker below the threshold is considered predictive of a positive response to therapy.

Table 2 Mutation Frequency Profile of Urothelial Carcinomas

Gene	UC, Bladder	UC, Nonbladder
APC	2/45 (4.4%)	0/16
BRAF	1/120 (0.8%)	0/24
CDH1	3/47 (6.4%)	0/16
c-KIT	1/78 (1.3%)	0/20
c-MET	1/47 (2.1%)	0/16
EGFR	1/53 (1.9%)	1/19 (5.3%)
ERBB2	1/47 (2.1%)	0/16
FBXW7	2/47 (4.3%)	2/16 (12.5%)
FGFR3	0/21	3/10 (30.0%)[a]
HNF1A	0/41	1/16 (6.3%)
HRAS	0/42	1/16 (6.3%)
KDR	1/47 (2.1%)	0/16
KRAS	5/135 (3.7%)	0/24
PIK3CA	19/113 (16.8%)	5/22 (22.7%)
PTEN	4/47 (8.5%)	1/16 (6.3%)
RB1	2/46 (4.3%)	0/16
SMAD4	1/47 (2.1%)	0/16
STK11	1/45 (2.2%)	0/16
TP53	23/46 (50.0%)	3/14 (21.4%)[a]

Abbreviation: UC = urothelial carcinoma.
[a]Significantly higher ($P < .05$) compared with bladder UC.

Expression of PTEN

The loss of PTEN protein expression was observed at a high rate in bladder (45 of 122), and nonbladder UC (13 of 30). Four of 47 bladder UCs and 1 of 16 nonbladder UCs harbored *PTEN* mutations. Two of the 4 bladder UC mutations were in the C2 domain, and 2 were in the phosphatase domain; the nonbladder UC mutation (phosphatase domain) has unknown significance.

Status of TOPO1 and TOPO2A (Anthracyclines)

Overexpression of TOPO2α protein was high in nonbladder UCs (41 of 57; 72%) and bladder UCs (246 of 361; 68%).

Gene amplification of *TOPO2A* was observed in 2 bladder UCs (4%), of which 1 also exhibited concurrent *HER2* amplification. In contrast, TOPO1 was more commonly overexpressed in bladder UC (24 of 381; 63%) than in nonbladder UC (24 of 62; 39%; $P < .0004$).

Steroid Receptors Profile (Antihormonal Therapy)

Androgen receptor was the most frequently expressed (69 of 426; 16%) steroid receptor in bladder UC (51 male, 18 female samples). ER and PR were overexpressed in 8 of 425 (4 female, 4 male samples; 2%) and 11 of 424 (7 male, 4 female samples; 3%) of the cases, respectively. Nonbladder UC exhibited similar positivity rates for AR, ER, and PR (4 of 65; 6%, 1 of 64; 2%, and 1 of 64; 2%, respectively).

Mutational Analysis

One hundred fifty-seven cases were sequenced, including 135 bladder UCs and 22 nonbladder UCs. Sixty-three cases were sequenced using the next-generation sequencing (NGS) panel; 94 were sequenced using Sanger sequencing. Mutations were identified in 19 genes (Table 2 and Supplemental Table 3). The most commonly mutated gene was *TP53*.

Mutations were identified in 51 bladder UC cases (38%; Table 2). Thirteen of these cases showed more than 1 mutation (including multiple mutations in a single gene), with 1 case, a metastatic UC to the lung, previously treated using adjuvant chemotherapy, exhibiting mutations in 4 genes (cadherin 1, type 1, E-cadherin [epithelial] [*CDH1*], *PTEN*, *PIK3CA*, and *TP53*). Of the remaining 12 cases, 1 case harbored 3 different mutations (*TP53*, *PTEN*, and *RB1*), and the remaining 11 cases had 2 mutations.

Common (> 5% rate) single mutations affecting bladder UC included *TP53* (23 of 46; 50%), *PIK3CA* (19 of 113; 17%), *PTEN* (4 of 47; 9%), and *CDH1* (3 of 47; 6%). No bladder UC exhibited an *FGFR3* mutation in contrast to the nonbladder UCs (3 of 10; $P = .02$). Of note, 2 of 3 nonbladder carcinomas (renal pelvis) with the *FGFR3* mutation represented high-grade, nonmuscle-infiltrating UC and the third case was a high-grade, invasive UC with invasion into renal sinus fat.

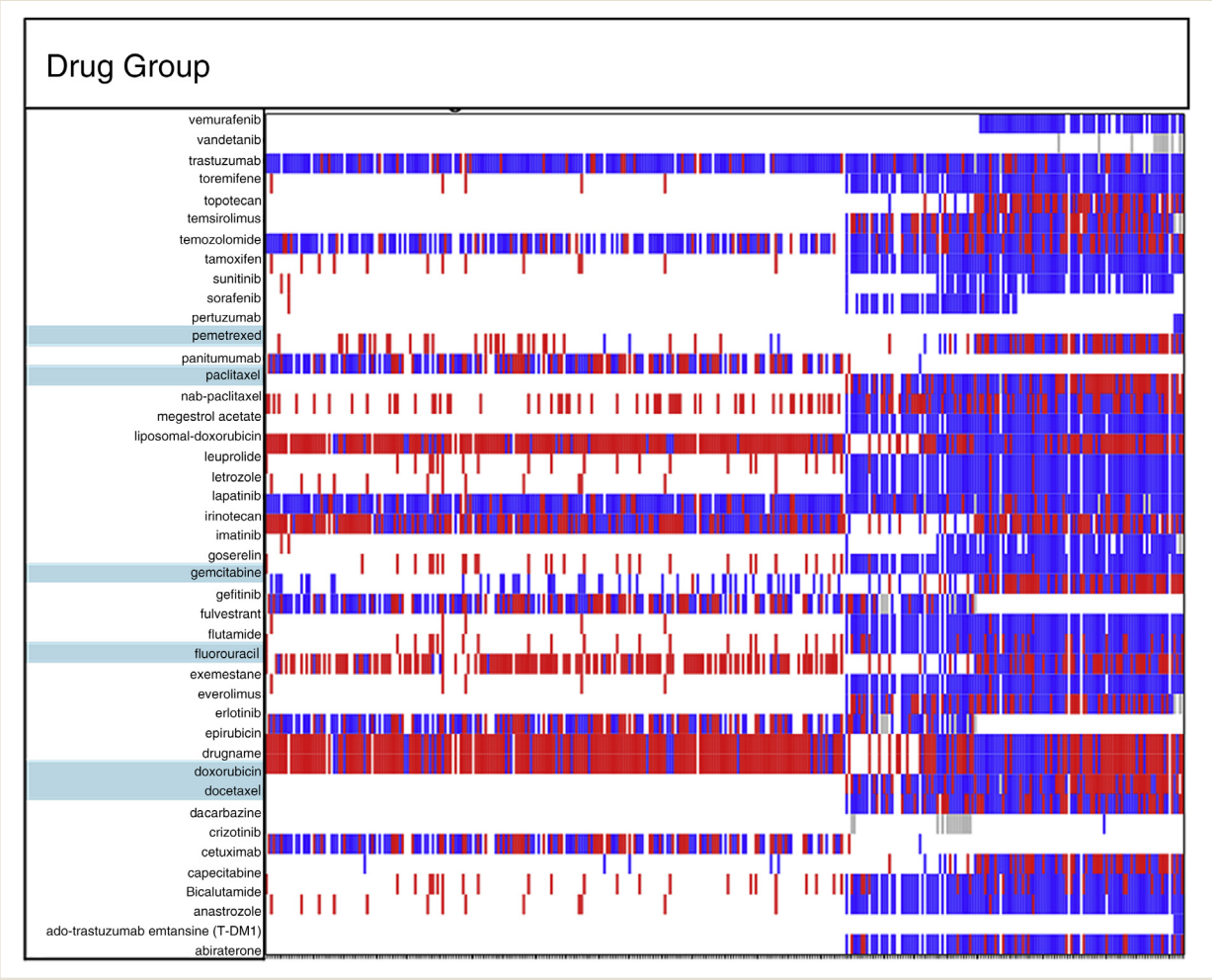
Four UCs of the bladder with *PIK3CA* mutations also showed concurrent mutations of *PTEN*, *TP53*, and *CDH1* (1 case), *TP53* (2 cases), and *KRAS* (1 case). All were HER2-negative. The most common *PIK3CA* mutations were E542K, E545K (helical domain), and H1074R (kinase domain) mutations present in 14 of 19 (74%) of the cases (Table 2). Adenomatous polyposis coli gene mutations were identified in 2 of 45 (4%) of the bladder cases of which 1 represented a nested variant of infiltrative bladder UC.

Of the nonbladder cases, mutations were identified in 10 of 15 cases tested with the NGS panel (69%; Supplemental Table 4). Multiple gene mutations were detected in 2 nonbladder UCs; both involved the F-box and WD repeat domain containing 7, E3 ubiquitin protein ligase (*FBXW7*) gene. Apart from the *FGFR3* (3 of 10; 30%) and *FBXW7* (2 of 16; 13%), nonbladder UCs showed patterns of mutations similar to their bladder counterparts (> 5%): *PTEN* (1 of 16; 6%), *TP53* (3 of 14; 21%), and *PIK3CA* (5 of 22; 23%). Single cases also harbored Harvey rat sarcoma viral oncogene homolog and *HNF1* homeobox *A* mutations that were not seen in UC of the bladder.

Biomarker—Drug Associations

The most common chemotherapeutic drugs associated with the potential benefit in UC were anthracyclines (overexpression of TOPO2α in 68% of bladder [246 of 361] and 72% of nonbladder UCs [41 of 57]). Other commonly associated drugs include mammalian target of rapamycin (mTOR) inhibitors (*PTEN* and *PIK3CA* alterations), TOPO1 inhibitors, and taxanes (overexpression of SPARC and TLE3; underexpression of TUBB3). Lack of potential benefit association was noted when a biomarker did not reach the threshold for the positive association or when a high expression of the biomarker led to a lack of potential benefit association (eg, TUBB3 and taxanes, MGMT and temozolomide/dacarbazine). The biomarker results for each case were associated with drugs with potential benefit or lack of potential benefit, based on the published literature. An overview of frequency of drug associations specific to the biomarker status of these cases is shown in

Figure 2 Drug Association Heat Map of Bladder Urothelial Carcinoma Using Caris Molecular Intelligence Recommendation Based on Biomarker Status and Published Level of Evidence



Highlighted Rows = National Comprehensive Cancer Network Recommendation. Red = Recommendation for Benefit; Gray = Indeterminate; and Blue = Recommendation for Lack of Benefit From Indicated Therapy

a heat map (Figure 2), revealing that many tumors express positive and negative predictive biomarkers and therapy associations, an important consideration in combination therapy.

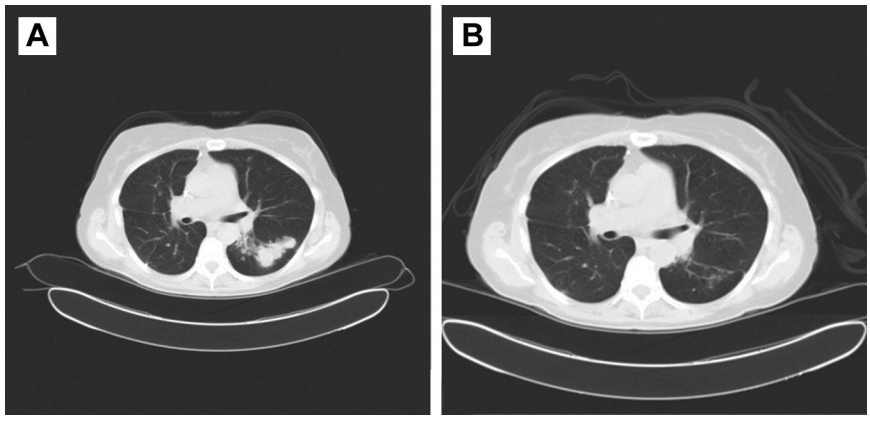
Biomarker–drug association benefit is best represented by patient outcomes. A single case study (Figure 3) provides an example of a HER2-positive patient treated with an anti-*HER2*/*neu* agent, with associated decrease in mass.

Discussion

Patients with locally advanced or metastatic UC are considered to have limited targeted therapeutic options. Standard chemotherapy treatment options are a combination of either methotrexate/vinblastine/adriamycin/cisplatin, or gemcitabin/cisplatin. Our multiplatform molecular survey of UC of bladder and nonbladder origin revealed 43 different therapeutic options across 98% of cases (based in large part on aberrations in biomarkers *PIK3CA, PTEN, EGFR, HER2,* TOPO2α, TOPO1, TUBB3, MGMT, and AR). In addition to increasing the potential options for therapies eightfold over standard care options, this approach results in an improvement in the proportion of cases (22% more) and a doubling in the number of drugs associated with potential benefit over a recently published study.[15]

Our study revealed frequent alterations of *PIK3CA* (mutations) and *PTEN* (protein loss with a few gene mutations) in UC. The spectrum of *PIK3CA* mutations differs from that seen in other cancers.[16] The most common *PIK3CA* mutations (E542K and E545K) affect the helical domain, and kinase domain mutations (eg, H1047R) are less common.[9,17] Of note, these mutations also characterize advanced nonurologic malignancies.[18] PTEN loss/inactivation has been implicated in UC development[13,16,19-23] and progression[24,25] and correlated with the tumor stage and grade.[7,16-17,19] *PTEN* mutations have been rarely identified in bladder UC. The PTEN status findings in bladder cancer are in line with the previous studies.[3,17,19,25-27] Taken together, our results indicate that deregulation of the *PIK3CA/PTEN* axis plays an important role in a subset of patients with locally advanced or

Figure 3 Chest Scan Images From a 62-Year-Old Female Patient With Metastatic Renal Pelvis Urothelial Carcinoma. The Patient was Found to be HER2/*neu* 2+ Positive Using Immunohistochemistry and had *HER2/neu* Amplification Using Fluorescence in Situ Hybridization (Ratio, 4.37). She Therefore Entered a Clinical Trial of Paclitaxel/Trastuzumab With the Investigational Anti-*HER2/neu* Agent MM 111. She was Treated With the Agents for 6 Months. These Images from the Chest Computed Tomography Scan are (A) Baseline and (B) After 2 Months of Therapy



metastatic UC that might be responsive to selective *PIK3CA/AKT/*mTOR inhibitors (eg, everolimus and temsirolimus).

Of the steroid receptors expression, the highest positivity rate was observed for AR, which has been correlated to better response to antiandrogen therapy in prostate, breast, and salivary gland cancers.[28-30] AR expression appears to progressively decrease with the tumor stage,[31] but we found AR expression in 16% of locally advanced/metastatic UC of the bladder, suggesting a significant potential for tailored therapy with antiandrogens in this specific subgroup.

HER2 status has been extensively studied in bladder UC with heterogeneous results on the frequency of *HER2* amplification (range, 0%-42%). The 2 largest studies conducted by Simon et al[12] and Lae et al[32] showed that the amplification rate of *HER2* is approximately 6%. Our study revealed that 12% of infiltrating UCs of the bladder harbored *HER2* amplification. The markedly higher *HER2* positivity in our study might be because of the predominantly advanced and metastatic UC we analyzed. Similarly, Fleischmann et al found substantially greater HER2 positivity (15%) in metastatic bladder cancers, compared with their primary counterparts (9%).[33] In contrast, the nonbladder UC cohort showed a rate of *HER2* positivity (6%) similar to the 2 previous studies.

HER2 amplification has been shown to be associated with improved response to HER2-targeted therapy such as trastuzumab, lapatinib, ado-trastuzumab, and pertuzumab in breast cancer[34,35] and advanced gastric/gastroesophageal junction and rectal cancers (trastuzumab).[36,37] Further, *HER2* status has been shown to influence clinical efficacy to anti-EGFR monoclonal antibodies in patients with metastatic colon cancer.[38] These results, along with the lack of the *PIK3CA/PTEN/AKT* alterations in most HER2-positive UCs of the bladder, indicate eligibility in a subset of patients for targeted therapy with trastuzumab or similar agents (Figure 3).

Despite the common TOPO2α protein expression in UC, *TOPO2*A amplification appears to be a rare event.[39-41] Increased expression of TOPO2α protein has been associated with histological responses and better disease free survival (DFS) to doxorubicin-based chemotherapy in soft tissue sarcoma patients.[42] Even though *TOPO2A* was not amplified in our samples, HER2 overexpression/amplification was noted and the vicinity of *TOPO2A* and *HER2* genes in chromosome 17q12[43]; the correlation between HER2 overexpression and response to anthracycline-containing therapies might suggest anthracyclines as a potential benefit to UC patients.

A DNA-repair gene involved in chemotherapy response, MGMT,[44] was commonly coexpressed in *EGFR*-amplified UC. In vivo and early phase clinical trials confirmed a therapeutic benefit for bladder patients when combined treatment based on EGFR inhibitors and taxanes was used.[5]

Expression of TUBB3 has been associated with the tumor invasiveness, poorly differentiated morphology, and decreased sensitivity to antitubulin agents such as taxanes and vinca alkaloids.[45] Our results, in line with previous data, indicate a potential resistance to antitubulin agents in a substantial proportion of UCs, particularly subsets of small cell carcinomas and adenocarcinomas of the bladder.

Epidermal growth factor receptor protein expression has been frequently observed in UC, and *EGFR* gene alterations were uncommon.[46-49] We found *EGFR* amplification in approximately 20% of bladder and nonbladder UCs. Thirty percent of those with *EGFR* amplification also harbored a *HER2* aberration. Combination therapy based on coactivation of *EGFR* and *HER2* in a subset of UC of the bladder might be promising because a previous clinical trial indicated a potential therapeutic benefit of such an approach in bladder patients with coactivation of these receptors.[5] In addition, the lack of *KRAS* alterations in EGFR-positive UC also supports the anti-EGFR therapeutic approach, observed in pancreatic cancer patients.[50] *EGFR* mutations, although rare, as earlier confirmed,[11] indicate a potential lack of response to EGFR tyrosine kinase inhibitors.

Another potential benefit of biomarker evaluation could be in the design of clinical trials for advanced UC. In 2 recent studies, responses to drugs with well-defined predictive biomarkers were investigated.[51,52] The studies could provide new combination therapies for metastatic UC, but unfortunately, the studies failed to evaluate molecular status of the patients. Inclusion of the biomarker status would allow patients to be stratified based on level of response.

Conclusion

Comprehensive molecular profiling of UC using multiple technologies has identified a number of actionable targets that could lead to individualized therapy using NCCN-recommended and therapies not currently approved for UC, but approved for other tumor types.

Clinical Practice Points

- Comprehensive molecular profiling of infiltrating UC might identify a large number of biomarker aberrations that might direct treatment using conventional chemotherapies and targeted therapies, not currently recommended in this population.
- As a group, bladder UCs exhibited higher levels of actionable biomarkers, suggesting that UCs from different primary sites and non-UCs are driven by different molecular pathways.

Disclosure

The authors have stated that they have no conflicts of interest.

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Supplemental Table 1	Immunohistochemistry
Antibody (Marker)	**Threshold**
Androgen Receptor (AR)	= 0+ or <10% or ≥1+ and ≥10%
c-Kit (CD117)	= 0+ and = 100% or ≥2+ and ≥30%
c-MET	<50% or <2+ or ≥2+ and ≥50%
Estrogen Receptor (ER)	= 0+ or <10% or ≥1+ and ≥10%
Progesterone Receptor (PR)	= 0+ or <10% or ≥1+ and ≥10%
Epidermal Growth Factor Receptor (EGFR)	2+ and ≥10%
Human Epidermal Growth Factor Receptor 2 (HER2)	≤1+ or = 2+ and ≤10% or ≥3+ and >10%
O(6)-methylguanine-methyltransferase (MGMT)	= 0+ or ≤35% or ≥1+ and >35%
P-Glycoprotein (PGP)	= 0+ or <10% or ≥1+ and ≥10%
Phosophatase and Tensin Homolog (PTEN)	= 0+ or ≤50% or ≥1+ and >50%
Ribonucleotide Reductase M1 (RRM1)	= 0+ or <50% or <2+ or ≥2+ and ≥50%
SPARC (Osteonectin)	<30% or <2+ or ≥2+ and ≥30%
Transducin-Like Enhancer of Split 3 (TLE3)	<30% or <2+ or ≥2+ and ≥30%
Topoisomerase 2 Alpha (TOPO2α)	= 0+ or <10% or ≥1+ and ≥10%
Topoisomerase 1 (TOPO1)	= 0+ or <30% or <2+ or ≥2+ and ≥30%
Thymydilate Synthase (TS)	= 0+ or ≤3+ and <10% or ≥1+ and ≥10%
Tubulin β-3 Chain (TUBB3)	<30% or <2+ or ≥2+ and ≥30%

Supplemental Table 2 Genes

Gene	Approved Symbol	Approved Name	HGNC ID	Location
MPL	MPL	Myeloproliferative leukemia virus oncogene	HGNC:7217	1p34
NRAS	NRAS	Neuroblastoma RAS viral (v-ras) oncogene homolog	HGNC:7989	1p13.2
ALK	ALK	Anaplastic lymphoma receptor tyrosine kinase	HGNC:427	2p23
IDH1	IDH1	Isocitrate dehydrogenase 1 (NADP+), soluble	HGNC:5382	2q32-qter
ERBB4	ERBB4	V-erb-b2 avian erythroblastic leukemia viral oncogene homolog 4	HGNC:3432	2q33.3-q34
VHL	VHL	Von Hippel-Lindau tumor suppressor, E3 ubiquitin protein ligase	HGNC:12687	3p25.3
MLH1	MLH1	MutL homolog 1, colon cancer, nonpolyposis type 2 (*E coli*)	HGNC:7127	3p22.3
CTNNB1	CTNNB1	Catenin (cadherin-associated protein), beta 1, 88kDa	HGNC:2514	3p21
PIK3CA	PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha	HGNC:8975	3q26.3
KDR	KDR	Kinase insert domain receptor (a type III receptor tyrosine kinase)	HGNC:6307	4q11-q12
KIT	KIT	V-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	HGNC:6342	4q11-q12
FBXW7	FBXW7	F-box and WD repeat domain containing 7, E3 ubiquitin protein ligase	HGNC:16712	4q31.23
APC	APC	Adenomatous polyposis coli	HGNC:583	5q21-q22
CSF1R	CSF1R	Colony stimulating factor 1 receptor	HGNC:2433	5q32
PDGFR	PDGFRB	Platelet-derived growth factor receptor, beta polypeptide	HGNC:8804	5q33.1
NPM1	NPM1	Nucleophosmin (nucleolar phosphoprotein B23, numatrin)	HGNC:7910	5q35.1
EGFR	EGFR	Epidermal growth factor receptor	HGNC:3236	7p12
MET	MET	Met proto-oncogene	HGNC:7029	7q31
SMO	SMO	Smoothened, frizzled family receptor	HGNC:11119	7q32.1
BRAF	BRAF	V-raf murine sarcoma viral oncogene homolog B	HGNC:1097	7q34
FGFR1	FGFR1	Fibroblast growth factor receptor 1	HGNC:3688	8p12
JAK2	JAK2	Janus kinase 2	HGNC:6192	9p24
ABL1	ABL1	C-abl oncogene 1, non-receptor tyrosine kinase	HGNC:76	9q34.1
NOTCH1	NOTCH1	Notch 1	HGNC:7881	9q34.3
RET	RET	Ret proto-oncogene	HGNC:9967	10q11.2
PTEN	PTEN	Phosphatase and tensin homolog	HGNC:9588	10q23
FGFR2	FGFR2	Fibroblast growth factor receptor 2	HGNC:3689	10q25.3-q26
HRAS	HRAS	Harvey rat sarcoma viral oncogene homolog	HGNC:5173	11p15.5
ATM	ATM	Ataxia telangiectasia mutated	HGNC:795	11q22-q23
KRAS	KRAS	Kirsten rat sarcoma viral oncogene homolog	HGNC:6407	12p12.1
PTPN11	PTPN11	Protein tyrosine phosphatase, non-receptor type 11	HGNC:9644	12q24.1
HNF1A	HNF1A	HNF1 homeobox A	HGNC:11621	12q24.31
FLT3	FLT3	Fms-related tyrosine kinase 3	HGNC:3765	13q12
RB1	RB1	Retinoblastoma 1	HGNC:9884	13q14.2
AKT1	AKT1	V-akt murine thymoma viral oncogene homolog 1	HGNC:391	14q32.32-q32.33
CDH1	CDH1	Cadherin 1, type 1, E-cadherin (epithelial)	HGNC:1748	16q22.1
TP53	TP53	Tumor protein	HGNC:11998	17p13.1
ERBB2	ERBB2	V-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2	HGNC:3430	17q11.2-q12
SMAD4	SMAD4	SMAD family member 4	HGNC:6770	18q21.1
GNA11	GNA11	Guanine nucleotide binding protein (G protein), alpha 11 (Gq class)	HGNC:4379	19p13.3
STK11	STK11	Serine/threonine kinase 11	HGNC:11389	19p13.3
JAK3	JAK3	Janus kinase 3	HGNC:6193	19p13-p12
GNAS	GNAS	GNAS complex locus	HGNC:4392	20q13.2-q13.3
SMARCB1	SMARCB1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1	HGNC:11103	22q11.23

Abbreviation: HGNC ID = HUGO Gene Nomenclature Committee unique gene ID.

Supplemental Table 3 Frequency and Types of Mutations Detected in Urinary Bladder Carcinomas

Gene	Number	P	PP	VUS
APC	2			2
K1454E				1
A1474T				1
BRAF	1	1		
G469A[a]	1	1		
CDH1	3			3
D400N	1			1
D402G	1			1
D402H	1			1
c-MET	1			1
T1010I	1			1
EGFR	3	3		
H773dup[a]	1	1		
770D_771NinsG	1	1		
G719S	1	1		
ERBB2	1			1
D769H	1			1
FBXW7	2		2	
R465L	1		1	
R479G	1		1	
FGFR3	3			
F384L		1		
S249C		2		
KDR	1			1
D1313H	1			1
c-KIT	1			1
G565V[a]	1			1
KRAS	5	5		
G12V	2	2		
G12A[a]	1	1		
G12R[a]	1	1		
G12S[a]	1	1		
PIK3CA	19	17	2	
E545K (7[a])	9	9		
E542K[a]	3	3		
H1047R (1)[a]	2	2		
Q546L[a]	2	2		
Q546R	1	1		
R88Q	2		2	
PTEN	4	3		1
L247fs	1	1		
R172S	1			1
T319X	1	1		
Y68H	1	1		
RB1	2			2
D571N	1			1
Y692C	1			1
SMAD4	1			1
T174N	1			1

Supplemental Table 3 Continued

Gene	Number	P	PP	VUS
STK11	1	1		
F354L	1	1		
TP53	23	17	5	1
D281N	1		1	
E171X	1	1		
E198X	1	1		
E271fs	1	1		
E271K	1		1	
E298X	1	1		
N239S	1		1	
P177L	1		1	
Q192X	1	1		
R158H	1		1	
R175H	2	2		
R196X	2	2		
R213P	1			1
R248Q	2	2		
R248W	2	2		
R306X	1	1		
S183X	2	2		
S241F	1	1		

Abbreviations: P = pathogenic; PP = presumed pathogenic; VUS = variant of unknown significance.
[a]Indicates identified by Sanger sequencing.

Supplemental Table 4 Mutation Frequency Profile of Different Subtypes of Nonurothelial Bladder Carcinoma

Gene	Small-Cell Carcinoma	Squamous Cell Carcinoma	Adenocarcinoma
APC	0/4	0/1	0/5
BRAF	0/9	0/3	0/7
CDH1	0/4	0/1	0/5
c-KIT	0/9	0/1	0/6
c-MET	0/4	0/1	0/5
EGFR	1/5 (20%; G719S)	0/1	0/5
ERBB2	0/4	0/1	0/5
FBXW7	0/4	0/1	0/5
FGFR3	0/3	0/1	0
HNF1A	0/2	0/1	0/4
HRAS	0/4	0/1	0/5
KDR	0/4	0/1	0/5
KRAS	0/11	0/3	5/9 (55.6%)[a]
PIK3CA	0/8	0/3	1/8 (12.5%)
PTEN	0/4	0/1	0/5
RB1	0/4	0/1	0/5
SMAD4	0/4	0/1	0/5
STK11	0/4	0/1	0/5
TP53	4/4 (100%)	0/1	1/4 (25%)

[a] $P < .001$ (compared with bladder urothelial carcinoma).